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IN THE UNITED STATES PATENT AND  
TRADEMARK OFFICE

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Applicants: John L. Schenk, George E. Seidel, Tae Kwang Suh  
Application Number:  
Filed:  
Title: Low Pressure Sperm Cell Separation System  
TC/A.U:  
Examiner:

International Application No: PCT/US2003/024460, Filed 01 August 2003  
Original US Application: 60/400,971, Filed 01 August 2002

Assignee: XY, Inc. and COLORADO STATE UNIVERSITY through its  
agent Colorado State University Research Foundation  
Attorney Docket: XY-LowPressure-USNP  
Customer No. 33549

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PRELIMINARY AMENDMENT

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**I. INTRODUCTORY REMARKS**

The claims have been amended to limit dependencies and to cancel claims 10-13, 17-20, 25-26, 28-29, 32, 34-35, 37-38 as filed in the original application and adding claims 63 and 64, leaving claims 1-9, 14-16, 21-24, 27, 30-31, 33, 36, and 39-64 remaining in the application. In the original application, a large number of claims were presented in order to adequately claim the various aspects of the present invention(s) in dependent and alternatively independent fashions. The applicants have voluntarily reduced the amount to assist the examiner in their efforts to primarily 3 independent and closely related claims (with a limited number of accompanying dependent claims) for a consideration in this first application without waiving the right to add claims such as dependent claims already disclosed herein or to file other applications such as continuations, divisionals, or continuations in part.

Before calculating filing fees and before examining the above-referenced application, please amend the application as follows:

**Amendments to the Specification** begin on page 3.

**Amendments to the Claims** begin on page 4 of this paper.

**Remarks** begin on page 16 of this paper.

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## II. AMENDMENTS TO THE SPECIFICATION:

Please replace paragraph 1 on page 1 of the specification with the following amended paragraph:

This application is the United States National Stage of International Patent Cooperation Treaty Patent Application No. PCT/US2003/024460, filed August 1, 2003 which claims the benefit of United States Provisional Patent Application No. 60/400,971, filed August 1, 2002, each hereby incorporated by reference herein.

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### III. AMENDMENTS TO THE CLAIMS:

*This listing of claims will replace all prior versions, and listings, of claims in the application (Section VI).*

#### **Listing of Claims:**

1. (Original): A method of generating a sperm cell insemination sample, comprising:
  - a. obtaining semen from a male of a species of mammal;
  - b. generating a fluid stream having flow characteristics;
  - c. altering flow characteristics of said fluid stream to adjust fluid stream pressure;
  - d. entraining said sperm cells into said fluid stream;
  - e. controlling sperm cell fertility characteristics through adjustment of said fluid stream pressure; and
  - f. generating a sperm cell insemination sample having controlled sperm cell fertility characteristics.
2. (Currently amended): A method of generating a sperm cell insemination sample as described in claim 1, ~~wherein~~ wherein said species of mammal is selected from the group consisting of a bovine species of mammal, an equine species of mammal, an ovine species of mammal, a canine species of mammal, a feline species of mammal, a swine species of mammal, a marine species of mammal, a deer species of mammal, a primate species of mammal, a goat species of mammal.
3. (Original): A method of generating a sperm cell insemination sample as described in claim 1, wherein said fluid stream comprises a sheath fluid stream.
4. (Original): A method of generating a sperm cell insemination sample as described in claim 3, wherein said sheath fluid stream comprises a sheath fluid containing phosphate buffered saline (PBS).

5. (Original): A method of generating a sperm cell insemination sample a described in claim 3, sheath fluid stream comprises a sheath fluid containing a citrate buffer.
6. (Original): A method of generating a sperm cell insemination sample a described in claim 5, wherein said citrate buffer comprises about 2.9% sodium citrate.
7. (Original): A method of generating a sperm cell insemination sample a described in claim 3, wherein said sheath fluid stream comprises a sheath fluid containing a HEPES buffer.
8. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said fluid stream is generated within a flow cytometer or cell sorter.
9. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises altering said flow characteristics to adjust said fluid stream pressure to between about 20 pounds per square inch and about 60 pounds per square inch.

Claims 10-13.

(Canceled)

14. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said bovine semen from said male of a bovine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to between about 30 pounds per square inch and about 50 pounds per square inch.

15. (Original): A method of generating a sperm cell insemination sample as described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said bovine semen from said male of a bovine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to between about 30 pounds per square inch and about 40 pounds per square inch.
16. (Original): A method of generating a sperm cell insemination sample as described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said bovine semen from said male of a bovine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to about 40 pounds per square inch.

Claims 17-20. (Canceled)

21. (Original): A method of generating a sperm cell insemination sample as described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to between about 30 pounds per square inch and about 50 pounds per square inch.
22. (Original): A method of generating a sperm cell insemination sample as described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid

stream pressure to between about 30 pounds per square inch and about 40 pounds per square inch.

23. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said step of obtaining semen from a male of a species of mammal comprises obtaining said equine semen from said male of a equine species of mammal and said step of altering flow characteristics of said fluid stream to adjust fluid stream pressure comprises adjusting said flow characteristics of said fluid stream to adjust fluid stream pressure to about 40 pounds per square inch.

24. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein one of said sperm cell fertility characteristics comprises sperm cell motility.

Claims 25-26. (Canceled)

27. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristic comprises sperm cell viability.

Claims 28-29. (Canceled)

30. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises pregnancy rate of a female of said species of mammal inseminated with said sperm cell insemination sample having controlled sperm cell fertility characteristics.

31. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises pregnancy rate of said female of said bovine species of mammal inseminated with a bovine sperm cell insemination sample having controlled sperm cell fertility characteristics.

32. (Canceled)

33. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises cleavage rate of oocytes fertilized with said sperm cell insemination sample having controlled sperm cell fertility characteristics.

Claims 34-35. (Canceled)

36. (Original): A method of generating a sperm cell insemination sample a described in claim 1, wherein said fertility characteristics comprises blastocyst rate of oocytes fertilized with said sperm cell insemination sample having controlled sperm cell fertility characteristics.

Claims 37-38. (Canceled)

39. (Original): A method of generating a sperm cell insemination sample a described in claim 31, wherein said bovine sperm cell insemination sample having controlled sperm cell fertility characteristics contains between about  $1 \times 10^5$  and  $2 \times 10^7$  of said bovine sperm cells.

40. (Original): A method of generating a sperm cell insemination sample a described in claim 31, wherein said bovine sperm cell insemination sample having controlled sperm cell fertility characteristics contains between about  $1 \times 10^6$  and  $3 \times 10^6$  of said bovine sperm cells.

41. (Original): A method of generating a sperm cell insemination sample a described in claim 1, further comprising the step of staining said sperm cells.



42. (Original): A method of generating a sperm cell insemination sample a described in claim 41, wherein said step of staining said sperm cells comprises staining said sperm cells with Hoechst 33342.
43. (Original): A method of generating a sperm cell insemination sample a described in claim 1, further comprising generating droplets in said fluid stream some of which contain one each of said sperm cells.
44. (Original): A method of generating a sperm cell insemination sample a described in claim 43, further comprising differentiating said sperm cells based upon a sex characteristic.
45. (Original): A method of generating a sperm cell insemination sample a described in claim 44, wherein said step of differentiating said sperm cells based on a sex characteristic comprises differentiating said sperm cells based on amount of DNA content.
46. (Original): A method of generating a sperm cell insemination sample a described in claim 44, wherein said step of differentiating said sperm cells based on a sex characteristic comprises differentiating said sperm cells based on sperm head volume.
47. (Original): A method of generating a sperm cell insemination sample a described in claim 44, further comprising the step of separating s sperm cells based on said sex characteristic.
48. (Original): A method of generating a sperm cell insemination sample a described in claim 47, further comprising the step of collecting said sperm cell insemination sample having controlled sperm cell fertility characteristics in a collection container.
49. (Original): A method of generating a sperm cell insemination sample a described in claim 48, wherein said step of collecting said sperm cell insemination sample having

controlled sperm cell fertility characteristics in a collection container comprises collecting a sex selected sperm cell insemination sample in said collection container.

50. (Original): A method of generating a sperm cell insemination sample a described in claim 49, wherein said sex selected sperm cell insemination sample comprises an artificial insemination sex selected sperm cell insemination sample.
51. (Original): A method of generating a sperm cell insemination sample a described in claim 49, wherein said sex selected sperm cell insemination sample comprises an in vitro fertilization sex selected sperm cell insemination sample.
52. (Original): A method of generating a sperm cell insemination sample a described in claim 49, wherein said sex selected sperm cell insemination sample comprises an intracytoplasmic sex selected sperm cell injection sample.
53. (Currently amended): A method of assessing sex selected sperm cell fertility, comprising:
- [[a.]] obtaining sperm cells from a male of a species of mammal;
  - [[b.]] providing Y-chromosome bearing sex selected sperm cells exposed to first treatment conditions;
  - [[c.]] providing X-chromosome bearing sex selected sperm cells exposed to second treatment conditions;
  - [[d.]] generating an insemination sample having a ratio of said Y-chromosome bearing sex selected sperm cells exposed to said first treatment conditions and said X-chromosome bearing sex selected sperm cells exposed to second treatment conditions;
  - [[e.]] inseminating at least one female of said species of mammal with said insemination sample having said ratio of said Y-chromosome bearing sperm cells exposed to said first treatment conditions and said X-chromosome bearing sperm cells exposed to second treatment conditions;
  - [[f.]] producing offspring from said at least one female of said species of mammal; and

[[g.]] assessing fertility of said sex selected sperm cells by comparison of sex ratio of offspring of said at least one female of said species of mammal to said ratio of said Y-chromosome bearing sperm cells exposed to first treatment conditions and X-chromosome bearing sperm cells exposed to second treatment ~~conditions~~ conditions.

54. (Original): A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said ratio of said Y-chromosome bearing sperm cells exposed to said first treatment conditions and said X-chromosome bearing sperm cells exposed to second treatment conditions comprises substantially equal numbers of Y-chromosome bearing sperm cells exposed to said first treatment conditions and said X-chromosome bearing sperm cells exposed to second treatment conditions.

55. (Original): A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said sex ratio of offspring of said at least one female of said species of mammal comprises a sex ratio of embryos.

56. (Original): A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said sex ratio of offspring of said at least one female of said species of mammal comprises a sex ratio of fetuses.

[[56.]] 57. (Currently amended): A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said sex ratio of offspring of said at least one female of said species of mammal comprises a sex ratio of offspring born.

[[57.]] 58. (Currently amended): A method of assessing sex selected sperm cell fertility as described in claim 53, wherein said at least one female of said species of said mammal comprises a sufficient number of females of said species to produce sufficient numbers of offspring to establish said sex ratio of offspring.

[[58.]] 59. (Currently amended): A method of assessing fertility of sperm cells from a male of a species of mammal, comprising:

- [[a.]] obtaining sperm cells from a least two males of a species of mammal;
- [[b.]] exposing said sperm cells from each of said at least two males of said species to substantially the same flow cytometric treatment;
- [[c.]] inseminating at least one female of said species of mammal with a mixture of substantially equal numbers of said sperm cells from each of said at least two males of said species of mammal exposed to substantially the same flow cytometric treatment;
- [[d.]] collecting embryos from said at least one female of said species of mammal;
- [[e.]] determining which of said at least two males of said species of mammal sired each embryo; and
- [[f.]] ranking comparative fertility of said at least two males of said species of mammal based on comparative number of embryos sired by each of said at least two males.

[[59.]] 60. (Currently amended): A method of assessing fertility of sperm cells from a male of a species of mammal as described in ~~claim 58~~ claim 59, wherein said flow cytometric treatment comprises separating said sperm cells from each of said at least two males based upon a sex characteristic.

[[60.]] 61. (Currently amended): A method of assessing fertility of sperm cells from a male of a species of mammal as described in ~~claim 58~~ claim 59, wherein said flow cytometric treatment comprises sex selecting sperm cells from each of said at least two males.

[[61.]] 62. (Currently amended): ~~A method of assessing fertility of sperm cells from a male of a species of mammal;~~ A method of generating a sperm cell insemination sample as described in claim 1 further comprising:

- [[a.]] obtaining sperm cells from a least two males of a species of mammal;
- [[b.]] exposing said sperm cells from each of said at least two males of said species to substantially the same flow cytometric treatment;

- [[c.]] fertilizing oocytes in vitro with a mixture of substantially equal numbers of said sperm cells from each of said at least two males of said species of mammal exposed to substantially the same flow cytometric treatment;
- [[d.]] collecting embryos generated through said in vitro fertilization of said oocytes;
- [[e.]] determining which of said at least two males of said species of mammal sired each embryo; and
- [[f.]] ranking comparative fertility of said at least two males of said species of mammal based on comparative number of embryos sired by each of said at least two males.

63. (New): A method of generating a sperm cell insemination sample as described in claim 1, further comprising:
- obtaining sperm cells from said male of said species of mammal;
  - providing Y-chromosome bearing sex selected sperm cells exposed to first treatment conditions;
  - providing X-chromosome bearing sex selected sperm cells exposed to second treatment conditions;
  - generating an insemination sample having a ratio of said Y-chromosome bearing sex selected sperm cells exposed to said first treatment conditions and said X-chromosome bearing sex selected sperm cells exposed to second treatment conditions;
  - inseminating at least one female of said species of mammal with said insemination sample having said ratio of said Y-chromosome bearing sperm cells exposed to said first treatment conditions and said X-chromosome bearing sperm cells exposed to second treatment conditions;
  - producing offspring from said at least one female of said species of mammal; and
  - assessing fertility of said sex selected sperm cells by comparison of sex ratio of offspring of said at least one female of said species of mammal to said ratio of said Y-chromosome bearing sperm cells exposed to first treatment conditions and X-chromosome bearing sperm cells exposed to second treatment conditions

64. (New): A method of generating a sperm cell insemination sample as described in claim 1, further comprising:
- obtaining sperm cells from at least two males of a species of mammal;
  - exposing said sperm cells from each of said at least two males of said species to substantially the same flow cytometric treatment;
  - inseminating at least one female of said species of mammal with a mixture of substantially equal numbers of said sperm cells from each of said at least two males of said species of mammal exposed to substantially the same flow cytometric treatment;
  - collecting embryos from said at least one female of said species of mammal;
  - determining which of said at least two males of said species of mammal sired each embryo; and
  - ranking comparative fertility of said at least two males of said species of mammal based on comparative number of embryos sired by each of said at least two males.

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#### IV. REMARKS

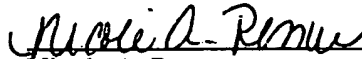
Preliminary amendments to limit the examination effort required for the claims have been made to submit certain embodiments of the present invention for examination in this initial application.

Importantly, it should be understood that the amendments submitted herein are made as a matter of practicality only, and should not to be construed as creating any situation of file wrapper estoppel or the like as all rights are expressly reserved and may be pursued in this or other applications, such as divisionals, continuations, or continuations-in-part if desired. Amendments are made for tangential issues of clarity and as a matter of the Office's convenience or expedience only. The amendments should not be interpreted as an action that in any way surrenders a particular equivalency, surrenders any right to patent coverage, or otherwise limits any rights that the Applicant may now or hereafter assert. It should be understood that, unless and to the extent deemed broadened by this amendment, and even as amended, the Applicant expressly reserves all rights, including but not limited to: all rights to maintain the scope of literal coverage with respect to any elements that may have existed under the language previously presented, all rights to maintain the scope of equivalency coverage as may have existed under the language previously presented, and all rights to re-present the prior language at any time in this or any subsequent application. To the extent currently foreseeable, no change or reduction in *direct or* equivalency coverage is believed to exist, and no change or reduction in *direct or* equivalency coverage is intended through the presentation of this amendment.

Claims 10-13, 17-20, 25-26, 28-29, 32, 34-35, and 37-38 have been cancelled. Applicant requests that claims 1-9, 14-16, 21-24, 27, 30-31, 33, 36, and 39-64 – each determined to have met the requirement for unity of invention during the international stage – be examined as a single group and allowed at the examiner's earliest convenience.

Dated this 31 day of January, 2004.

Respectfully submitted,



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IN THE UNITED STATES PATENT AND  
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Applicants: John L. Schenk, George E. Seidel, Tae Kwang Suh  
Application Number: 10/523,268  
317 Date: July 7, 2005  
Title: Low Pressure Sperm Cell Separation System  
TC/A.U: 1632  
Examiner: Marcia Stephens Noble  
Assignee: XY, Inc.  
Attorney Docket: XY-LowPressure-USNP  
Confirmation No. 5591  
Customer No. 33549

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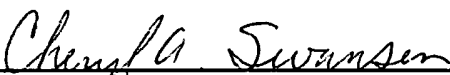
**CERTIFICATE OF EXPRESS MAILING**

I, Cheryl A. Swanson, hereby certify to the truth of the following items:

1. I am an employee of Santangelo Law Offices, P.C., 125 South Howes, Third Floor, Fort Collins, Colorado 80521.

2. I have this day deposited the attached true and correct copy of the Preliminary Amendment filed January 31, 2005 as downloaded from the USPTO PAIRS website with a "Copy Only" watermark added with the United States Postal Service as Express Mail, postage prepaid, for mailing to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.

Dated this 28<sup>th</sup> day of November, 2006.

  
Cheryl A. Swanson